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<110> New England Biolabs, Inc.  
Maina, Claude V.  
Tzertzinis, George  
Kumar, Sanjay

<120> Compositions and Methods for Generating Short Double-Stranded RNA  
Using Mutated RNase III

<130> NEB-238-PUS

<150> PCT/US05/02029

<151> 2005-01-21

<150> 60/538,805

<151> 2004-01-23

<150> 60/572,240

<151> 2004-05-18

<150> 60/543,880

<151> 2004-02-12

<160> 26

<170> PatentIn version 3.2

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Met Thr Gln Asn Leu Glu Arg Leu Gln Arg Gln Ile Gly Tyr Gln Phe  
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Asn Gln Pro Ala Leu Leu Lys Gln Ala Leu Thr His Arg Ser Ala Ala  
20 25 30

Val Lys His Asn Glu Arg Leu Glu Phe Leu Gly Asp Ala Ile Leu Asn  
35 40 45

Phe Ile Ile Ala Glu Ala Leu Tyr His Gln Phe Pro Lys Cys Asn Glu  
50 55 60

Gly Glu Leu Ser Arg Met Arg Ala Thr Leu Val Arg Glu Pro Thr Leu  
65 70 75 80

Ala Ser Leu Ala Arg Gln Phe Glu Leu Gly Asp Tyr Leu Ser Leu Gly  
85 90 95

Pro Gly Glu Leu Lys Ser Gly Gly Phe Arg Arg Glu Ser Ile Leu Ala  
100 105 110

Asp Cys Val Glu Ala Ile Ile Gly Ala Ile Ser Leu Asp Ser Asp Leu  
115 120 125

Ala Thr Thr Thr Lys Ile Val Gln His Trp Tyr Gln Ala Gln Leu Lys  
130 135 140

Gln Ile Gln Pro Gly Asp Asn Gln Lys Asp Pro Lys Thr Arg Leu Gln  
145 150 155 160

Glu Tyr Leu Gln Gly Lys Arg Leu Pro Leu Pro Thr Tyr Asn Val Val  
165 170 175

Glu Ile Lys Gly Glu Ala His Cys Gln Thr Phe Thr Val Glu Cys Tyr  
180 185 190

Val Lys Asn Ile Asp Arg Thr Phe Met Gly Ser Gly Ala Ser Arg Arg  
195 200 205

Lys Ala Glu Gln Ala Ala Ala Glu Lys Ile Leu Gln Leu Leu Glu Met  
210 215 220

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Lys  
225

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<223> relevant region from H. Influenzae

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Met Asn His Leu Asp Arg Leu Glu Arg Lys Ile Gly Tyr Arg Phe Asn  
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Asp Ile Ala Leu Leu Lys Gln Ala Leu Thr His Arg Ser Ala Ala Thr  
20 25 30

Gln His Asn Glu Arg Leu Glu Phe Leu Gly Asp Ser Ile Leu Asn Phe  
35 40 45

Thr Ile Ala Glu Ala Leu Tyr His Gln Phe Pro Arg Cys Asn Glu Gly  
50 55 60

Glu Leu Ser Arg Met Arg Ala Thr Leu Val Arg Glu Pro Thr Leu Ala  
65 70 75 80

Ile Leu Ala Arg Gln Phe Glu Leu Gly Asp Tyr Met Ser Leu Gly Ser  
85 90 95

Gly Glu Leu Lys Asn Gly Gly Phe Arg Arg Glu Ser Ile Leu Ala Asp  
100 105 110

Cys Val Glu Ala Ile Ile Gly Ala Met Ser Leu Asp Gln Gly Leu Ala  
115 120 125

Val Thr Thr Gln Val Ile Arg Asn Trp Tyr Gln Gln Leu Leu Ala Glu  
130 135 140

Ile Lys Pro Gly Asp Asn Gln Lys Asp Ala Lys Thr Arg Leu Gln Glu  
145 150 155 160

Tyr Leu Gln Gly Lys His Leu Pro Leu Pro Thr Tyr Glu Val Val Asn  
165 170 175

Ile Gln Gly Glu Ala His Cys Gln Ile Phe Thr Val Lys Cys Lys Val  
180 185 190

Lys Ser Ala Glu Lys Ile Asp Arg Thr Phe Val Ala Lys Gly Ser Ser  
 195 200 205

Arg Arg Lys Ala Glu Gln Ala Ala Ala Glu Gln Ile Leu Lys Glu Leu  
 210 215 220

Asp Ile Lys  
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<210> 11  
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<400> 11

Met Asn Pro Ile Val Ile Asn Arg Leu Gln Arg Lys Leu Gly Tyr Thr  
 1 5 10 15

Phe Asn His Gln Glu Leu Leu Gln Gln Ala Leu Thr His Arg Ser Ala  
 20 25 30

Ser Ser Lys His Asn Glu Arg Leu Glu Phe Leu Gly Asp Ser Ile Leu  
 35 40 45

Ser Phe Val Ile Ala Asn Ala Leu Ser Arg Phe Pro Arg Val Asp Glu  
 50 55 60

Gly Asp Met Ser Arg Met Arg Asp Pro Leu Val Arg Gly Asn Thr Leu  
 65 70 75 80

Ala Glu Leu Ala Arg Glu Phe Asp Leu Gly Glu Cys Leu Arg Leu Gly  
 85 90 95

Pro Gly Glu Leu Lys Ser Gly Gly Phe Arg Arg Glu Ser Ile Leu Ala  
 100 105 110

Asp Thr Val Glu Ala Leu Ile Gly Gly Val Phe Leu Asp Ser Asn Ile  
 115 120 125

Gln Thr Val Glu Gln Leu Ile Leu Asn Trp Tyr Lys Thr Arg Leu Asp  
 130 135 140

Glu Ile Ser Pro Gly Asp Lys Gln Lys Asp Pro Lys Thr Arg Leu Gln  
 145 150 155 160

Glu Tyr Leu Gln Gly Arg His Leu Pro Leu Pro Ser Tyr Leu Val Val  
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165

175

Gln Val Arg Gly Glu Ala His Asp Gln Glu Phe Thr Ile His Cys Gln  
180 185 190

Val Ser Gly Leu Ser Glu Pro Val Val Gly Thr Gly Ser Ser Arg Arg  
195 200 205

Lys Ala Glu Gln Ala Ala Ala Asn Ser Val Lys Lys Leu Glu Leu Glu  
210 215 220

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<400> 12

Met Asn Pro Ile Val Ile Asn Arg Leu Gln Arg Lys Leu Gly Tyr Thr  
1 5 10 15

Phe Asn His Gln Glu Leu Leu Gln Gln Ala Leu Thr His Arg Ser Ala  
20 25 30

Ser Ser Lys His Asn Glu Arg Leu Glu Phe Leu Gly Asp Ser Ile Leu  
35 40 45

Ser Tyr Val Ile Ala Asn Ala Leu Tyr His Arg Phe Pro Arg Val Asp  
50 55 60

Glu Gly Asp Met Ser Arg Met Arg Ala Thr Leu Val Arg Gly Asn Thr  
65 70 75 80

Leu Ala Glu Leu Ala Arg Glu Phe Glu Leu Gly Glu Cys Leu Arg Leu  
85 90 95

Gly Pro Gly Glu Leu Lys Ser Gly Gly Phe Arg Arg Glu Ser Ile Leu  
100 105 110

Ala Asp Thr Val Glu Ala Leu Ile Gly Gly Val Phe Leu Asp Ser Asp  
115 120 125

Ile Gln Thr Val Glu Lys Leu Ile Leu Asn Trp Tyr Gln Thr Arg Leu  
130 135 140

Asp Glu Ile Ser Pro Gly Asp Lys Gln Lys Asp Pro Lys Thr Arg Leu  
145 150 155 160

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Gln Glu Tyr Leu Gln Gly Arg His Leu Pro Leu Pro Thr Tyr Leu Val  
165 170 175

Val Gln Val Arg Gly Glu Ala His Asp Gln Glu Phe Thr Ile His Cys  
180 185 190

Gln Val Ser Gly Leu Ser Glu Pro Val Val Gly Thr Gly Ser Ser Arg  
195 200 205

Arg Lys Ala Glu Gln Ala Ala Ala Glu Gln Ala Leu Lys Lys Leu Glu  
210 215 220

Leu Glu  
225

<210> 13  
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<212> PRT  
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<220>  
<223> relevant region from V. cholerae

<400> 13

Met Thr Pro Pro Met Asn Lys Leu Thr Ser Lys Leu Gly Tyr Thr Phe  
1 5 10 15

Lys Glu Thr Glu Leu Leu Asn Leu Ala Leu Thr His Arg Ser Ala Asn  
20 25 30

Gly Lys His Asn Glu Arg Leu Glu Phe Leu Gly Asp Ser Ile Leu Ser  
35 40 45

Phe Val Ile Ala Asp Glu Leu Tyr Arg Arg Phe Pro Lys Val Asn Glu  
50 55 60

Gly Asp Met Ser Arg Met Arg Ala Thr Leu Val Arg Gly Asn Thr Leu  
65 70 75 80

Ala Glu Leu Gly Arg Glu Phe Asp Leu Gly Asp Tyr Leu Lys Leu Gly  
85 90 95

Pro Gly Glu Leu Lys Ser Gly Gly Phe Arg Arg Asp Ser Ile Leu Ala  
100 105 110

Asp Ala Val Glu Ala Ile Ile Gly Ala Ile Tyr Leu Asp Ser Asp Leu  
115 120 125

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Glu Thr Ala Arg Ser Ile Val Leu Glu Trp Tyr His Gly Arg Leu Glu  
130 135 140

Glu Ile Lys Pro Gly Ala Ser Gln Lys Asp Pro Lys Thr Arg Leu Gln  
145 150 155 160

Glu Phe Leu Gln Gly Arg Arg Lys Pro Leu Pro Val Tyr Thr Val Thr  
165 170 175

Asn Ile Lys Gly Glu Ala His Asn Gln Glu Phe Thr Val Ala Cys Glu  
180 185 190

Val Ala Gly Met Asp Thr Pro Val Ile Gly Lys Gly Thr Ser Arg Arg  
195 200 205

Lys Ala Glu Gln Ala Ala Ala Glu Thr Ala Leu Glu Gln Leu Thr Asn  
210 215 220

Gly  
225

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<400> 14

Met Ser Asn Ser Leu Asp Arg Leu Glu Arg Lys Leu Gly Tyr Thr Phe  
1 5 10 15

Lys Asp Arg Asp Leu Met Val Leu Ala Leu Thr His Arg Ser Tyr Ala  
20 25 30

Gly Arg Asn Asn Glu Arg Leu Glu Phe Leu Gly Asp Ala Ile Leu Asn  
35 40 45

Phe Val Ile Gly Glu Ala Leu Phe His His Phe Pro Gln Ala Arg Glu  
50 55 60

Gly Gln Leu Ser Arg Leu Arg Ala Arg Leu Val Lys Gly Glu Thr Leu  
65 70 75 80

Ala Leu Leu Ala Arg Gly Phe Glu Val Gly Asp Tyr Leu Arg Leu Gly  
85 90 95



Ser Gly Glu Leu Lys Ser Gly Gly Phe Arg Arg Glu Ser Ile Leu Ala  
100 105 110

Asp Ala Met Glu Ala Leu Ile Gly Ala Ile Tyr Leu Asp Thr Gly Met  
115 120 125

Asp Ser Ala Arg Glu Arg Ile Ile Ala Trp Leu Gly Pro Gln Leu Arg  
130 135 140

Glu Leu Thr Pro Val Asp Thr Asn Lys Asp Pro Lys Thr Arg Leu Gln  
145 150 155 160

Glu Phe Leu Gln Ser Arg Gly Cys Asp Leu Pro Arg Tyr Glu Val Val  
165 170 175

Asp Ile Gln Gly Glu Pro His Cys Arg Thr Phe Phe Val Asp Cys Glu  
180 185 190

Val Ala Leu Leu Ser Asp Lys Thr His Gly His Gly Gly Ser Arg Arg  
195 200 205

Ile Ala Glu Gln Val Ala Ala Ala Ala Ala Leu Val Ala Leu Gly Val  
210 215 220

Glu Asn Gly His Asp  
225

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<223> relevant region from H. pylori

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Met Lys Asn Lys Arg Ser Gln Asn Ser Pro Tyr Val Thr Pro Asp Asn  
1 5 10 15

Pro Tyr Leu Thr Leu Glu Lys Ala Leu Gly Tyr Ser Phe Lys Asp Lys  
20 25 30

Arg Leu Leu Glu Gln Ala Leu Thr His Lys Ser Cys Lys Leu Ala Leu  
35 40 45

Asn Asn Glu Arg Leu Glu Phe Leu Gly Asp Ala Val Leu Gly Leu Val  
50 55 60

Ile Gly Glu Leu Leu Tyr His Lys Phe Tyr Gln Tyr Asp Glu Gly Lys  
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65                      70                      75                      80  
 Leu Ser Lys Leu Arg Ala Ser Ile Val Ser Ala His Gly Phe Thr Lys  
                                  85                      90                      95  
 Leu Ala Lys Ala Ile Ala Leu Gln Asp Tyr Leu Arg Val Ser Ser Ser  
                                  100                      105                      110  
 Glu Glu Ile Ser Lys Gly Arg Glu Lys Pro Ser Ile Leu Ser Ser Ala  
                                  115                      120                      125  
 Phe Glu Ala Leu Met Ala Gly Val Tyr Leu Glu Ala Gly Leu Ala Lys  
                                  130                      135                      140  
 Val Arg Lys Ile Ile Gln Asn Leu Leu Asn Arg Ala Tyr Lys Arg Leu  
                                  145                      150                      155                      160  
 Asp Leu Glu His Leu Phe Met Asp Tyr Lys Thr Ala Leu Gln Glu Leu  
                                  165                      170                      175  
 Thr Gln Ala Gln Phe Cys Val Ile Pro Thr Tyr Gln Leu Leu Gln Glu  
                                  180                      185                      190  
 Lys Gly Pro Asp His His Lys Glu Phe Glu Met Ala Leu Tyr Ile Gln  
                                  195                      200                      205  
 Asp Lys Met Tyr Ala Thr Ala Lys Gly Lys Ser Lys Lys Glu Ala Glu  
                                  210                      215                      220  
 Gln Gln Cys Ala Tyr Gln Ala Leu Gln Lys Leu Lys Glu Ala Lys  
                                  225                      230                      235  
  
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 Asn Asp Leu Thr Leu Leu Glu Thr Ala Phe Thr His Thr Ser Tyr Ala  
                                  20                      25                      30  
 Asn Glu His Arg Leu Leu Asn Val Ser His Asn Glu Arg Leu Glu Phe  
                                  35                      40                      45

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Leu Gly Asp Ala Val Leu Gln Leu Ile Ile Ser Glu Tyr Leu Phe Ala  
50 55 60

Lys Tyr Pro Lys Lys Thr Glu Gly Asp Met Ser Lys Leu Arg Ser Met  
65 70 75 80

Ile Val Arg Glu Glu Ser Leu Ala Gly Phe Ser Arg Phe Cys Ser Phe  
85 90 95

Asp Ala Tyr Ile Lys Leu Gly Lys Gly Glu Glu Lys Ser Gly Gly Arg  
100 105 110

Arg Arg Asp Thr Ile Leu Gly Asp Leu Phe Glu Ala Phe Leu Gly Ala  
115 120 125

Leu Leu Leu Asp Lys Gly Ile Asp Ala Val Arg Arg Phe Leu Lys Gln  
130 135 140

Val Met Ile Pro Gln Val Glu Lys Gly Asn Phe Glu Arg Val Lys Asp  
145 150 155 160

Tyr Lys Thr Cys Leu Gln Glu Phe Leu Gln Thr Lys Gly Asp Val Ala  
165 170 175

Ile Asp Tyr Gln Val Ile Ser Glu Lys Gly Pro Ala His Ala Lys Gln  
180 185 190

Phe Glu Val Ser Ile Val Val Asn Gly Ala Val Leu Ser Lys Gly Leu  
195 200 205

Gly Lys Ser Lys Lys Leu Ala Glu Gln Asp Ala Ala Lys Asn Ala Leu  
210 215 220

Ala Gln Leu Ser Glu Val  
225 230

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Met Lys Glu Leu Gln Thr Val Leu Lys Asn His Phe Ala Ile Glu Phe  
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Ala Asp Lys Lys Leu Leu Glu Thr Ala Phe Thr His Thr Ser Tyr Ala  
20 25 30

Asn Glu His Arg Leu Leu Lys Ile Ser His Asn Glu Arg Leu Glu Phe  
35 40 45

Leu Gly Asp Ala Val Leu Gln Leu Leu Ile Ser Glu Tyr Leu Tyr Lys  
50 55 60

Lys Tyr Pro Lys Lys Pro Glu Gly Asp Leu Ser Lys Leu Arg Ala Met  
65 70 75 80

Ile Val Arg Glu Glu Ser Leu Ala Gly Phe Ala Arg Asp Cys Gln Phe  
85 90 95

Asp Gln Phe Ile Lys Leu Gly Lys Gly Glu Glu Lys Ser Gly Gly Arg  
100 105 110

Asn Arg Asp Thr Ile Leu Gly Asp Ala Phe Glu Ala Phe Leu Gly Ala  
115 120 125

Leu Leu Leu Asp Lys Asp Val Ala Lys Val Lys Glu Phe Ile Tyr Gln  
130 135 140

Val Met Ile Pro Lys Val Glu Ala Gly Glu Phe Glu Met Ile Thr Asp  
145 150 155 160

Tyr Lys Thr His Leu Gln Glu Leu Leu Gln Val Asn Gly Asp Val Ala  
165 170 175

Ile Arg Tyr Gln Val Ile Ser Glu Thr Gly Pro Ala His Asp Lys Val  
180 185 190

Phe Asp Val Glu Val Leu Val Glu Gly Lys Ser Ile Gly Gln Gly Gln  
195 200 205

Gly Arg Ser Lys Lys Leu Ala Glu Gln Glu Ala Ala Lys Asn Ala Val  
210 215 220

Glu Lys Gly Leu Asp Ser Cys Ile  
225 230

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<212> PRT  
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<223> relevant region from *B. subtilis*

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Met Ser Lys His Ser His Tyr Lys Asp Lys Lys Lys Phe Tyr Lys Lys  
 1 5 10 15

Val Glu Gln Phe Lys Glu Phe Gln Glu Arg Ile Ser Val His Phe Gln  
 20 25 30

Asn Glu Lys Leu Leu Tyr Gln Ala Phe Thr His Ser Ser Tyr Val Asn  
 35 40 45

Glu His Arg Lys Lys Pro Tyr Glu Asp Asn Glu Arg Leu Glu Phe Leu  
 50 55 60

Gly Asp Ala Val Leu Glu Leu Thr Ile Ser Arg Phe Leu Phe Pro Lys  
 65 70 75 80

Tyr Pro Ala Met Ser Glu Gly Asp Leu Thr Lys Leu Arg Ala Ala Ile  
 85 90 95

Val Cys Glu Pro Ser Leu Val Ser Leu Ala His Glu Leu Ser Phe Gly  
 100 105 110

Asp Leu Val Leu Leu Gly Lys Gly Glu Glu Met Thr Gly Gly Arg Lys  
 115 120 125

Arg Pro Ala Leu Leu Ala Asp Val Phe Glu Ala Phe Ile Gly Ala Leu  
 130 135 140

Tyr Leu Asp Gln Gly Leu Glu Pro Val Glu Ser Phe Leu Lys Val Tyr  
 145 150 155 160

Val Phe Pro Lys Ile Asn Asp Gly Ala Phe Pro His Val Met Asp Phe  
 165 170 175

Lys Ser Gln Leu Gln Glu Tyr Val Gln Arg Asp Gly Lys Gly Ser Leu  
 180 185 190

Glu Tyr Lys Ile Ser Asn Glu Lys Gly Pro Ala His Asn Arg Glu Phe  
 195 200 205

Glu Ala Ile Val Ser Leu Lys Gly Glu Pro Leu Gly Val Gly Asn Gly  
 210 215 220

Arg Ser Lys Lys Glu Ala Glu Gln His Ala Ala Gln Glu Ala Leu Ala  
 225 230 235 240

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Lys Leu Glu Lys His His Thr Lys Gln Leu Asn Pro Pro Tyr Asp Ser  
245 250 255

Gly Gly Phe Gln Tyr Val Cys Arg Leu Ile  
260 265

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<223> relevant region from S. aureus

<400> 19

Met Ser Lys Gln Lys Lys Ser Glu Ile Val Asn Arg Phe Arg Lys Arg  
1 5 10 15

Phe Asp Thr Lys Met Thr Glu Leu Gly Phe Thr Tyr Gln Asn Ile Asp  
20 25 30

Leu Tyr Gln Gln Ala Phe Ser His Ser Ser Phe Ile Asn Asp Phe Asn  
35 40 45

Met Asn Arg Leu Asp His Asn Glu Arg Leu Glu Phe Leu Gly Asp Ala  
50 55 60

Val Leu Glu Leu Thr Val Ser Arg Tyr Leu Phe Asp Lys His Pro Asn  
65 70 75 80

Leu Pro Glu Gly Asn Leu Thr Lys Met Arg Ala Thr Ile Val Cys Glu  
85 90 95

Pro Ser Leu Val Ile Phe Ala Asn Lys Ile Gly Leu Asn Glu Met Ile  
100 105 110

Leu Leu Gly Lys Gly Glu Glu Lys Thr Gly Gly Arg Thr Arg Pro Ser  
115 120 125

Leu Ile Ser Asp Ala Phe Glu Ala Phe Ile Gly Ala Leu Tyr Leu Asp  
130 135 140

Gln Gly Leu Asp Ile Val Trp Lys Phe Ala Glu Lys Val Ile Phe Pro  
145 150 155 160

His Val Glu Gln Asn Glu Leu Leu Gly Val Val Asp Phe Lys Thr Gln  
165 170 175

Phe Gln Glu Tyr Val His Gln Gln Asn Lys Gly Asp Val Thr Tyr Asn  
 180 185 190

Leu Ile Lys Glu Glu Gly Pro Ala His His Arg Leu Phe Thr Ser Glu  
 195 200 205

Val Ile Leu Gln Gly Glu Ala Ile Ala Glu Gly Lys Gly Lys Thr Lys  
 210 215 220

Lys Glu Ser Glu Gln Arg Ala Ala Glu Ser Ala Tyr Lys Gln Leu Lys  
 225 230 235 240

Gln Ile Lys

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 <223> relevant region from Borella burgdorferi

<400> 20

Met Met Lys Lys Lys Ser Ser Asp Phe Cys Leu Cys Asn Glu Arg Lys  
 1 5 10 15

Ser Gln Leu Ser Lys Phe Leu Glu Asn Leu Ser Ile Asp Phe Ser Asn  
 20 25 30

Phe Asp Leu Leu Asn Thr Ala Leu Cys His Ser Ser Tyr Ser Asn Glu  
 35 40 45

Leu Asp Gln Lys Ser Ser Asn Asn Glu Arg Leu Glu Phe Leu Gly Asp  
 50 55 60

Ser Val Leu Asn Leu Ile Ile Thr Asp His Leu Tyr Lys Thr Tyr Pro  
 65 70 75 80

Asn Lys Ser Glu Gly Glu Leu Ser Lys Ala Arg Ser Tyr Ile Val Ser  
 85 90 95

Glu Asp Ser Leu Ser Asn Ile Ala Arg Glu Ile Asn Leu Gly Ser Tyr  
 100 105 110

Ile Leu Leu Gly Arg Gly Glu Glu Ser Asn Asp Gly Arg Asn Lys Lys  
 115 120 125

Gly Ile Leu Ala Asp Ala Ile Glu Ala Phe Val Gly Ala Ile Tyr Leu  
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135

Asp Ser Gly Phe Ser Arg Ala Thr Glu Phe Val Val Gly Leu Phe Asp  
145 150 155 160

Met Tyr Ile Arg Leu Met Phe Asn Arg Gly Asp Phe Lys Asp Tyr Lys  
165 170 175

Ser Leu Leu Gln Glu Tyr Val Gln Lys Lys Tyr Lys Ile Ser Pro Ser  
180 185 190

Tyr Lys Leu Asp Lys Glu Ile Gly Pro Asp His Asp Lys Val Phe Cys  
195 200 205

Val Glu Leu Tyr Val Gly Glu Asn Phe Ile Ser Asn Gly Lys Gly Lys  
210 215 220

Ser Lys Lys Glu Ala Glu Met Arg Ala Ala Glu Val Ala Leu Lys Ala  
225 230 235 240

Met Glu Asn Ile Asn Leu  
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<211> 238  
<212> PRT  
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<220>  
<223> relevant region from M. leprae

<400> 21

Met Thr Gln Pro Arg Gln Ala Leu Leu Asp Ala Phe Gly Val Asp Leu  
1 5 10 15

Pro Asp Glu Leu Leu Ser Leu Ala Leu Thr His Arg Ser Tyr Ala Tyr  
20 25 30

Glu His Gly Gly Leu Pro Thr Asn Glu Arg Leu Glu Phe Leu Gly Asp  
35 40 45

Ala Val Leu Ser Leu Thr Ile Thr Asp Glu Leu Phe His Arg His Pro  
50 55 60

Asp Arg Ser Glu Gly Asp Leu Ala Lys Leu Arg Ala Ser Val Val Asn  
65 70 75 80

Thr Gln Ala Leu Ala Tyr Val Ala Arg Asn Leu Ser Asp Gly Gly Leu  
85 90 95



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Gly Val Tyr Leu Leu Leu Gly Arg Gly Glu Thr Asn Thr Gly Gly Ala  
100 105 110

Asp Lys Ser Ser Ile Leu Ala Asp Gly Met Glu Ser Leu Leu Gly Ala  
115 120 125

Ile Tyr Leu His His Gly Ile Glu Val Ala Arg Gln Val Ile Leu Arg  
130 135 140

Leu Phe Gly Thr Leu Leu Asp Ala Ala Pro Thr Leu Gly Ala Gly Leu  
145 150 155 160

Asp Trp Lys Thr Ser Leu Gln Glu Leu Thr Ala Ala Arg Gly Met Gly  
165 170 175

Val Pro Ser Tyr Val Val Thr Ser Thr Gly Pro Asp His Asp Lys Glu  
180 185 190

Phe Thr Ala Val Val Val Val Met Asp Thr Glu Tyr Gly Ser Gly Ile  
195 200 205

Gly His Ser Lys Lys Glu Ala Glu Gln Lys Ala Ala Ser Ala Ala Trp  
210 215 220

Lys Ala Leu Asp Val Leu Gly Gly Val Gly Lys Thr Ser Val  
225 230 235

<210> 22  
<211> 221  
<212> PRT  
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<220>  
<223> relevant region from Aquifex aeolicus

<400> 22

Met Lys Met Leu Glu Gln Leu Glu Lys Lys Leu Gly Tyr Thr Phe Lys  
1 5 10 15

Asp Lys Ser Leu Leu Glu Lys Ala Leu Thr His Val Ser Tyr Ser Lys  
20 25 30

Lys Glu His Tyr Glu Thr Leu Glu Phe Leu Gly Asp Ala Leu Val Asn  
35 40 45

Phe Phe Ile Val Asp Leu Leu Val Gln Tyr Ser Pro Asn Lys Arg Glu  
50 55 60

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Gly Phe Leu Ser Pro Leu Lys Ala Tyr Leu Ile Ser Glu Glu Phe Phe  
65 70 75 80

Asn Leu Leu Ala Gln Lys Leu Glu Leu His Lys Phe Ile Arg Ile Lys  
85 90 95

Arg Gly Lys Ile Asn Glu Thr Ile Ile Gly Asp Val Phe Glu Ala Leu  
100 105 110

Trp Ala Ala Val Tyr Ile Asp Ser Gly Arg Asp Ala Asn Phe Thr Arg  
115 120 125

Glu Leu Phe Tyr Lys Leu Phe Lys Glu Asp Ile Leu Ser Ala Ile Lys  
130 135 140

Glu Gly Arg Val Lys Lys Asp Tyr Lys Thr Ile Leu Gln Glu Ile Thr  
145 150 155 160

Gln Lys Arg Trp Lys Glu Arg Pro Glu Tyr Arg Leu Ile Ser Val Glu  
165 170 175

Gly Pro His His Lys Lys Lys Phe Ile Val Glu Ala Lys Ile Lys Glu  
180 185 190

Tyr Arg Thr Leu Gly Glu Gly Lys Ser Lys Lys Glu Ala Glu Gln Arg  
195 200 205

Ala Ala Glu Glu Leu Ile Lys Leu Leu Glu Glu Ser Glu  
210 215 220

<210> 23  
<211> 227  
<212> PRT  
<213> unknown

<220>  
<223> relevant region from Rickettsia conorii

<400> 23

Met Glu Ser Phe Glu Lys Leu Glu Lys Leu Leu Ser Tyr Ser Phe Lys  
1 5 10 15

Asn Lys Glu Leu Leu Ile Glu Ala Leu Ser His Pro Ser Leu Arg Gln  
20 25 30

His His Glu Tyr Lys Asp Asp Lys Asp Tyr Glu Arg Leu Glu Phe Leu  
35 40 45

Gly Asp Ala Val Leu Asn Leu Val Ile Thr Glu Ile Leu Phe Arg Asn  
 50 55 60

Phe Ala Asn Tyr Asn Glu Gly Asn Leu Ala Lys Ile Arg Ser Tyr Leu  
 65 70 75 80

Val Cys Lys Glu Thr Ile Cys Met Val Gly Ala Lys Leu Thr Leu Lys  
 85 90 95

Asn Tyr Ile Ile Met Thr His Gly Glu Glu Val Ala Gly Gly Arg Asp  
 100 105 110

Asn Leu Asn Asn Ile Glu Asn Ala Thr Glu Ala Leu Ile Ala Ala Ile  
 115 120 125

Tyr Leu Asp Ser Asn Ile Glu Thr Thr His Asp Ile Ile Glu Asn Leu  
 130 135 140

Trp Ala Glu Phe Ile Lys Val Gln Asn Leu Thr Asp Tyr Asp Pro Lys  
 145 150 155 160

Thr Ala Leu Gln Glu Trp Ala Gln Ala Ser Asp His His Leu Pro Ile  
 165 170 175

Tyr Arg Leu Ile Lys Arg Glu Gly Ala Ser His Ser Ser Thr Phe Thr  
 180 185 190

Val Leu Val Lys Val Lys Asp Tyr Glu Gln Thr Gly Thr Gly His Thr  
 195 200 205

Ile Lys Glu Ala Glu Lys Asn Ala Ala Arg Ser Leu Leu His Arg Leu  
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Lys Asn Asp  
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Gly Met Ser Lys Thr Lys Pro Leu Ser Ala Asp Glu Ile Ser Arg Leu  
 Page 19

Glu Ala Leu Ile Gly Tyr Glu Phe Lys Glu Lys Ala Arg Leu Asp Arg  
35 40 45

Ala Leu Thr His Ala Ser Ala Arg Ser Ala Ala Ala Gly Asn Tyr Glu  
50 55 60

Arg Leu Glu Phe Leu Gly Asp Arg Val Leu Gly Leu Cys Val Ala Glu  
65 70 75 80

Leu Leu Phe Ser Thr Phe Arg Asn Ala Ser Glu Gly Glu Leu Ser Val  
85 90 95

Arg Leu Asn Gln Leu Val Ser Ala Glu Ser Cys Ala Ala Ile Gly Asp  
100 105 110

Glu Met Gly Leu His Asn Phe Ile Arg Thr Gly Ser Asp Val Lys Lys  
115 120 125

Leu Thr Gly Lys Ala Met Leu Asn Val Arg Ala Asp Val Val Glu Ser  
130 135 140

Leu Ile Ala Thr Leu Tyr Leu Asp Gly Gly Leu Glu Ala Ser Arg Lys  
145 150 155 160

Phe Ile Leu Lys Tyr Trp Gln Gly Arg Ala Thr Ser Val Asp Ala Gly  
165 170 175

Arg Arg Asp Ala Lys Thr Glu Leu Gln Glu Trp Ala His Ala Arg Phe  
180 185 190

Ala Ala Thr Pro Ala Tyr Arg Val Asp Asp Arg Ser Gly Pro Asp His  
195 200 205

Asp Pro Ser Phe Thr Val Thr Val Glu Ile Pro Gly Val Lys Pro Glu  
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Leu Asp Asn Glu Asn Gly Ser Gln Gln Arg Glu Asn Ile Asn Thr Lys  
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Thr Leu Leu Lys Gly Asn Leu Lys Ile Ser Asn Tyr Lys Tyr Leu Glu  
 35 40 45

Val Ile Gln Leu Glu His Ala Val Thr Lys Leu Val Glu Ser Tyr Asn  
 50 55 60

Lys Ile Ile Glu Leu Ser Pro Asn Leu Val Ala Tyr Asn Glu Ala Val  
 65 70 75 80

Asn Asn Gln Asp Arg Val Pro Val Gln Ile Leu Pro Ser Leu Ser Arg  
 85 90 95

Tyr Gln Leu Lys Leu Ala Ala Glu Leu Lys Thr Leu His Asp Leu Lys  
 100 105 110

Lys Asp Ala Ile Leu Thr Glu Ile Thr Asp Tyr Glu Asn Glu Phe Asp  
 115 120 125

Thr Glu Gln Lys Gln Pro Ile Leu Gln Glu Ile Ser Lys Ala Asp Met  
 130 135 140

Glu Lys Leu Glu Lys Leu Glu Gln Val Lys Arg Glu Lys Arg Glu Lys  
 145 150 155 160

Ile Asp Val Asn Val Tyr Glu Asn Leu Asn Glu Lys Glu Asp Glu Glu  
 165 170 175

Glu Asp Glu Gly Glu Asp Ser Tyr Asp Pro Thr Lys Ala Gly Asp Ile  
 180 185 190

Val Lys Ala Thr Lys Trp Pro Pro Lys Leu Pro Glu Ile Gln Asp Leu  
 195 200 205

Ala Ile Arg Ala Arg Val Phe Ile His Lys Ser Thr Ile Lys Asp Lys  
 210 215 220

Val Tyr Leu Ser Gly Ser Glu Met Ile Asn Ala His Asn Glu Arg Leu  
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|            |                      |            |            |            |            |            |            |            |            |            |            |            |            |            |            |  |
|------------|----------------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|--|
| 225        | NEB-238-PUS.ST25.txt |            |            |            |            |            |            |            |            |            | 235        | 240        |            |            |            |  |
| Glu        | Phe                  | Leu        | Gly        | Asp<br>245 | Ser        | Ile        | Leu        | Asn        | Ser<br>250 | Val        | Met        | Thr        | Leu        | Ile<br>255 | Ile        |  |
| Tyr        | Asn                  | Lys        | Phe<br>260 | Pro        | Asp        | Tyr        | Ser        | Glu<br>265 | Gly        | Gln        | Leu        | Ser        | Thr<br>270 | Leu        | Arg        |  |
| Met        | Asn                  | Leu<br>275 | Val        | Ser        | Asn        | Glu        | Gln<br>280 | Ile        | Lys        | Gln        | Trp        | Ser<br>285 | Ile        | Met        | Tyr        |  |
| Asn        | Phe<br>290           | His        | Glu        | Lys        | Leu        | Lys<br>295 | Thr        | Asn        | Phe        | Asp        | Leu<br>300 | Lys        | Asp        | Glu        | Asn        |  |
| Ser<br>305 | Asn                  | Phe        | Gln        | Asn        | Gly<br>310 | Lys        | Leu        | Lys        | Leu        | Tyr<br>315 | Ala        | Asp        | Val        | Phe        | Glu<br>320 |  |
| Ala        | Tyr                  | Ile        | Gly        | Gly<br>325 | Leu        | Met        | Glu        | Asp        | Asp<br>330 | Pro        | Arg        | Asn        | Asn        | Leu<br>335 | Pro        |  |
| Lys        | Ile                  | Arg        | Lys<br>340 | Trp        | Leu        | Arg        | Lys        | Leu<br>345 | Ala        | Lys        | Pro        | Val        | Ile<br>350 | Glu        | Glu        |  |
| Ala        | Thr                  | Arg<br>355 | Asn        | Gln        | Val        | Ala        | Leu<br>360 | Glu        | Lys        | Thr        | Asp        | Lys<br>365 | Leu        | Asp        | Met        |  |
| Asn        | Ala<br>370           | Lys        | Arg        | Gln        | Leu        | Tyr<br>375 | Ser        | Leu        | Ile        | Gly        | Tyr<br>380 | Ala        | Ser        | Leu        | Arg        |  |
| Leu<br>385 | His                  | Tyr        | Val        | Thr        | Val<br>390 | Lys        | Lys        | Pro        | Thr        | Ala<br>395 | Val        | Asp        | Pro        | Asn        | Ser<br>400 |  |
| Ile        | Val                  | Glu        | Cys        | Arg<br>405 | Val        | Gly        | Asp        | Gly        | Thr<br>410 | Val        | Leu        | Gly        | Thr        | Gly<br>415 | Val        |  |
| Gly        | Arg                  | Asn        | Ile<br>420 | Lys        | Ile        | Ala        | Gly        | Ile<br>425 | Arg        | Ala        | Ala        | Glu        | Asn<br>430 | Ala        | Leu        |  |
| Arg        | Asp                  | Lys<br>435 | Lys        | Met        | Leu        | Asp        | Phe<br>440 | Tyr        | Ala        | Lys        | Gln        | Arg<br>445 | Ala        | Ala        | Ile        |  |
| Pro        | Arg<br>450           | Ser        | Glu        | Ser        | Val        | Leu<br>455 | Lys        | Asp        | Pro        | Ser        | Gln<br>460 | Lys        | Asn        | Lys        | Lys        |  |
| Arg<br>465 | Lys                  | Phe        | Ser        | Asp        | Thr<br>470 | Ser        |            |            |            |            |            |            |            |            |            |  |

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